THE PRESENT STATUS OF LIVER FUNCTION TESTS INCLUDING OBSERVATIONS ON THE NEWER FLOCCULATION PROCEDURES*†

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THE liver is an organ with many functions and a large number of tests have been devised to evaluate clinically these divers functions. A classification of the tests based on the various physiological functions of the liver described by Greene and Bruger in 1943[1] has been revised and brought up to date. This classification is presented in Table I; the more important tests according to the concept of the present authors are capitalized.

In the following pages, a brief resumé of the present status of some of these liver function tests will be presented. In the strict sense of the term, the determination of serum bilirubin or of urine urobilinogen is not a test for evaluating the functional capacity of the liver but these laboratory procedures are included in this discussion because of their importance in the diagnosis of liver and biliary tract disease.

Bilirubin in the Serum: The most accurate index of jaundice is the quantitative measurement of bilirubin in the serum. Normal values using the method of Thannhauser and Andersen[2] (or any of its modifications) do not exceed 1.0 mg. per cent. The method of Malloy and Evelyn[3] utilizing a photo-electric colorimeter has a normal range not exceeding 0.8 mg. per cent. Recently, Ducci and Watson[4] recommended the determination of the prompt direct reacting bilirubin; normal values are not in excess of 0.2 mg. per cent.

Bilirubin in the Urine: During an epidemic of infectious hepatitis

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in the recent World War, Gellis and Stokes[5] utilized a modified methylene blue test to detect bilirubin in the urine. They found this procedure of value in the pre-icteric stage of infectious hepatitis. The test is simple to perform and may be used as a screen in the early detection of hepatic involvement as a result of exposure to certain industrial poisons. The reliability of the methylene blue reaction, however, has been questioned[6] since the test is not specific for bilirubin; yellow urines from normal persons and yellow substances such as potassium dichromate or ferric chloride will produce a green color when added to methylene blue.[7] The Harrison spot test[8] and the barium strip modification[9] may also be used to detect bilirubinuria and, for the most part, these procedures have replaced the older tests such as those of Rosenbach, Gmelin, etc.

Icterus Index: This does not have the specificity of the quantitative bilirubin since it merely measures the intensity of yellow color in the serum as compared with an arbitrary standard. The standard most commonly used is the color of a 1:50,000 solution of potassium dichromate. Any substance that will impart yellow color to the serum will elevate the icterus index. Thus, increased amounts of carotene, nicotinic acid, riboflavin and traces of hemoglobin may give false readings. Lipemic sera are also difficult to read and give false values. As a rough clinical guide, the icterus index has its place, but in cases where the presence or absence of jaundice is in doubt, the quantitative serum bilirubin is the test of choice.

Van Den Bergh Reaction: This color reaction forms the basis for the determination of quantitative serum bilirubin. The indirect and direct van den Bergh reactions do not differentiate between hepatocellular and obstructive jaundice since both reactions are present in these disorders. In the differential diagnosis of jaundice, its main value lies in the detection of hemolytic icterus since in this type of jaundice only an indirect reaction is obtained.

The form in which bilirubin exists in the blood stream probably accounts for the type of response obtained. Bilirubinglobin derived from the breakdown of hemoglobin in the cells of the reticulo-endothelial system gives the indirect reaction whereas bilirubin resulting from the breakdown of bilirubinglobin in the parenchymal cells of the liver produces the direct reaction. It follows, therefore, that a direct van den Bergh reaction is obtained in both hepatocellular and ob-

TABLE I.—CLASSIFICATION OF LIVER FUNCTION TESTS

I. *The Excretory Functions of the Liver*

A. *Formation and Secretion of Bile*

1. Secretion of bile acids
   a. Concentration of bile acids in blood
2. Excretion of bile pigments
   a. Bilirubin
      (I) Concentration of bilirubin in bile
      (II) Retention of bilirubin in blood
         A. QUANTITATIVE SERUM BILIRUBIN
         B. ICTERUS INDEX
         C. VAN DEN BERGH
            (1) Bilirubinglobin (indirect reaction)
            (2) Bilirubin (direct reaction)
      (III) Excretion of Bilirubin in urine
         A. Rosenbach's nitric acid method
         B. Iodine ring test
         C. METHYLENE BLUE REACTION
         D. HARRISON SPOT TEST
         E. BARIUM CHLORIDE STRIP METHOD
   b. UROBILIN AND UROBILINOGEN IN URINE
   c. Porphyrin (the urinary/fecal coproporphyrin ratio)
3. Excretion of cholesterol (concentration of cholesterol in bile)
4. Excretion of inorganic salts
   a. Sodium Chloride
   b. Calcium
5. Excretion of water

B. *Excretory Loading Tests*

1. Bilirubin
   a. Bilirubin excretion test
2. Dyes
   a. Phenoltetrachlorphthalein excretion test
   b. Rose-Bengal excretion test
   c. Azorubin-S excretion test
   d. BROMSULPHALEIN EXCRETION TEST
   e. Phenoltetraiodophthalein excretion test

II.I *The Metabolic Functions of the Liver*

A. *Carbohydrate Metabolism*

1. Fasting blood sugar
2. GALACTOSE TOLERANCE TEST
   a. ORAL
   b. Intravenous
3. Dextrose tolerance test
4. Fructose tolerance test
5. Utilization of d-lactic acid

(Continued on next page)

TABLE I.—*Continued from preceding page*

B. *Protein Metabolism*
   1. Concentration of amino-acids in blood
   2. Concentration of urea in blood
   3. Concentration of ammonia in blood
   4. Concentration of uric acid in blood
   5. Concentration of guanidine in blood
   6. Tyrosine tolerance test
   7. Methionine clearance test

C. *Fat Metabolism*
   1. Concentration of cholesterol in blood
      a. TOTAL CHOLESTEROL
      b. CHOLESTEROL PARTITION (ESTER/TOTAL RATIO)
   2. Fat tolerance tests
      a. Oral
      b. Intravenous

D. *Blood Forming Functions*
   1. Erythrocytes (Macrocytic hyperchromic anemia)
   2. Plasma proteins
      a. Fibrinogen
      b. Albumin and globulins
         (I) CONCENTRATION OF SERUM ALBUMIN
         (II) Takata-Ara reaction
         (III) Formol-gel reaction
         (IV) Weltmann coagulation band
         (V) Magnesium chloride test
         (VI) Colloidal gold reaction
         (VII) CEPHALIN-CHOLESTEROL FLOCCULATION
         (VIII) THYMOL TURBIDITY
         (IX) THYMOL FLOCCULATION
         (X) COLLOIDAL RED PRECIPITATION
         (XI) ZINC SULPHATE PRECIPITATION
      c. Prothrombin
         (I) PROTHROMBIN TIME
         (II) RESPONSE OF PROTHROMBIN TIME TO VITAMIN K ADMINISTRATION

E. *Detoxification Function*
   1. HIPPURIC ACID SYNTHESIS
      a. ORAL
      b. INTRAVENOUS
   2. Cincophen test

F. *Phosphatase Production*
   1. CONCENTRATION OF SERUM ALKALINE PHOSPHATASE

structive jaundice since in either condition bilirubin is regurgitated into the blood stream. The positive indirect response obtained in these disorders is due to the normal amount of circulating bilirubinglobin. In hemolytic icterus, there is no regurgitation of bilirubin but an excess of bilirubinglobin is present in the blood, hence a strongly positive indirect reaction alone is obtained.

Urobilin and Urobilinogen in Urine: Urobilinogen is formed in the small intestine by bacterial reduction of bilirubin. Part is excreted in the feces while the remainder is re-absorbed into the blood stream. The major part of this fraction is returned to the liver for re-excretion, while the rest is eliminated by the kidneys. It follows, therefore, that bile must enter the small bowel for urobilinogen to be produced. In complete obstruction of the extrahepatic type, there is a persistent absence of urobilinogen in the urine. In incomplete extrahepatic obstruction, the urinary urobilinogen varies between very low to normal or slightly above normal values. In hepatocellular jaundice, the urinary urobilinogen is low or absent in the acute phase followed by a gradual increase even above normal during recovery. This late increase is due to the inability of the liver to re-excrete that portion of urobilinogen which is returned to it via the enterohepatic circulation. In jaundice due to cholangiolitis, the urine urobilinogen may be absent in the early stages; thereafter, the values tend to be within normal limits. In our hands, the modified 2-hour test as described by Watson[10] has proved to be a satisfactory procedure for the quantitative estimation of urobilinogen in the urine. It should be emphasized that serial determinations of urinary urobilinogen are frequently necessary to establish the presence or absence of bile in the gastro-intestinal tract.

Bromsulphalein Excretion: Several dyes have been used clinically to test the excretory functional capacity of the liver. Of these, bromsulphalein[11] has won the widest acceptance. It is a sensitive and safe procedure with the added advantage of simplicity. The dye is removed from the blood stream by the Kupffer cells of the liver and then excreted by the parenchymal cells into the bile. Five milligrams of dye per kilogram of body weight is the usual dose given. Specimens of blood are then taken at 30 minutes and 60 minutes as recommended by O'Leary, Greene and Rowntree,[12] or a single specimen at 45 minutes as recommended by Mateer.[13] Using the latter, a normal liver should clear all the dye in 45 minutes so that none remains in the

serum. Values up to 4 per cent retention are considered normal if a photo-electric colorimeter is used instead of the usual comparator block. This test, however, is of very limited value in the presence of jaundice.

Galactose Tolerance: This test, introduced by Bauer,[14] is the one of choice in estimating the carbohydrate function of the liver. Unlike glucose, it does not require insulin for its metabolism and so far as is known, has no renal threshold. It has the added advantage of being rapidly absorbed. In the oral test, 40 grams of galactose dissolved in 400 ml. of water are taken with the subject in a fasting state, the urine collected for 5 hours and a quantitative estimation for urine sugar done. Normally, no more than 3 grams of sugar are excreted in the 5 hour period. The presence of impaired renal function introduces considerable error.

The galactose tolerance test is positive in acute and chronic parenchymal disease of the liver. It may be used in the presence of jaundice as an aid in differentiating parenchymal and obstructive jaundice. Bensley[15] reported a positive oral galactose test in 474 cases of toxic and infectious jaundice; in 210 cases of obstructive jaundice, the test was positive in 19 per cent of cases.

Bassett, Althausen and Coltrin[16] have developed an intravenous galactose tolerance test. In our opinion, the simpler oral test provides as much information as can be derived from the more complicated intravenous procedure.

Total Cholesterol and Cholesterol Partition: The value for total cholesterol in the blood plasma of normal adults varies between 160 and 230 mg. per cent. The cholesterol esters vary between 60 and 120 mg. per cent. The ratio of the combined to the total cholesterol is relatively constant ranging between 40 and 52 per cent when the Bloor[17] or modified Bloor techniques are used.

Thannhauser and Schaber,[18] Epstein and Greenspan,[19] Hurxthal and Hunt,[20] Klein[21] and Greene, Hotz and Leahy[22] have shown that in the presence of severe parenchymal damage to the liver, the cholesterol esters in the blood were reduced or entirely absent. Moreover, these workers noted that a reduction of cholesterol esters was seen more frequently in parenchymatous than in obstructive jaundice, this decrease being a better indication of the severity of the damage than as an aid in differential diagnosis.

Elevation of the total plasma or serum cholesterol occurs in obstructive jaundice of both the intrahepatic (cholangiolitic) and extrahepatic types. In most instances, the total cholesterol remains unchanged in the parenchymal forms of jaundice.

Serum Albumin: Since albumin is formed exclusively in the liver, it is not surprising that the concentration of this protein fraction in the blood is decreased in certain forms of liver disease. The normal level of serum albumin is 4 to 5 gm. per 100 ml. Tumen and Bockus[23] found that a reduction in albumin was the most consistent alteration in the serum proteins in patients with chronic advanced liver disease. Lowering of the albumin-globulin ratio was not found to be as significant or as constant as the reduction in serum albumin. Post and Patek[24] noted a rise in serum albumin during clinical improvement in patients with cirrhosis of the liver. Persistently low or decreasing serum albumin concentration was a poor prognostic sign.

Flocculation Tests: The flocculation tests are not strictly liver function tests. These procedures determine qualitative and quantitative alterations of various fractions of the serum proteins which result from disturbed activity of the parenchymal cells of the liver.

Cephalin-Cholesterol Flocculation: This test was proposed by Hanger in 1938.[25] He showed that in patients with active parenchymal disease of the liver, the serum flocculated a cephalin-cholesterol emulsion. Since then, many studies have confirmed this. Positive cephalin-cholesterol flocculation has also been reported in new-born infants with jaundice,[26] in cases of hyperthyroidism,[27] malaria,[28] in catatonics and schizophrenics[29] and in many other clinical disorders.

It is believed that gamma globulin is the protein fraction that takes an active part in the flocculation of the cephalin-cholesterol emulsion.[30] Under normal circumstances, the serum albumin has the capacity to inhibit the flocculating action of gamma globulin. In cases of parenchymal liver disease, the serum albumin fraction has a decreased capacity to inhibit this flocculating action on the part of the gamma globulin.[31] Readings of 2+ to 4+ are considered abnormal.

Thymol Turbidity and Flocculation: In 1944, Maclagan[32] described a new test of liver dysfunction utilizing a saturated aqueous solution of thymol buffered with barbital and sodium barbital at a pH of 7.8. He obtained essentially the same results with this thymol solution as with colloidal gold solution; in favor of the former was the ease with

which the solution could be prepared. Subsequent work confirmed the value of the thymol turbidity test as an indicator of abnormal activity of the liver parenchyma. The mechanism, however, is unlike that of the cephalin-cholesterol flocculation test.[33,34] Kunkel and Hoagland[35] showed that the turbidity of the thymol solution produced by certain sera depended on the presence of lipids and some abnormal lipo-protein associated with the beta globulin fraction of the serum. The gamma globulin fraction of the serum also played a role in the reaction. The relative importance of the different components in the reaction varied with different sera. As a general rule, in acute hepatitis cephalin-cholesterol flocculation becomes positive earlier than the thymol turbidity test while the latter remains positive longer during convalescence. Due to this fact and in view of the different underlying mechanisms, the thymol turbidity and cephalin-cholesterol tests supplement rather than supplant each other.[36]

Methods for quantitatively determining the degree of thymol turbidity produced by sera using various photo-electric colorimeters have been reported.[37,38] However, the original method reported by Maclagan, utilizing the formazin standards devised by Kingsbury an associates,[39] provides a simple and clinically accurate method. By this procedure, normal readings are 0 to 4 units.

In his original description of the thymol turbidity test, Maclagan stated that when the test was positive, flocculation frequently occurred in the tubes allowed to stand overnight; he felt, however, that this was not an essential part of the test. Neefe[40] noted that thymol flocculation remained positive for some time after the thymol turbidity reaction had returned to the normal range. In addition to supplementing and possibly increasing the sensitivity of the thymol turbidity test, the presence of 2+to 4+ flocculation at 18 hours or its absence may aid in evaluating the significance of borderline or weakly positive thymol turbidity readings.

Colloidal Red Test: Ducci[41] has recently recommended this procedure in which a colloidal suspension of scarlet red is used. The test is performed in the same manner as Maclagan's colloidal gold reaction. It is a sensitive and simple procedure and appears to parallel the cephalin-cholesterol flocculation and thymol turbidity tests. Positive flocculations probably depend on alterations in the serum gamma globulin fraction.

Zinc Sulphate Precipitations: Kunkel[42] reported that when serum with abnormally high gamma globulin concentration was diluted with a solution containing a small amount of zinc sulphate, a turbid precipitate formed and the optical density of the suspension was proportional to the concentration of gamma globulin. Normal serum did not flocculate for at least 12 hours while serum from patients with even slight elevation of gamma globulin fraction usually flocculated within 4 hours. While the test was not specific and was positive in cases having hypergammaglobulinemia from causes other than liver disease, it was found useful in determining the alterations in gamma globulin during the course of infectious hepatitis. Kunkel stated that the test was of particular value in detecting persistent liver disease following infectious hepatitis. In a group of 41 patients with cirrhosis of the liver, the reaction was positive in each case.

Prothrombin Time: The production of prothrombin by the liver is dependent upon ingested vitamin K. Bile salts are necessary for the absorption of vitamin K from the small bowel. In liver disease, the ability of the parenchymal cells to produce prothrombin may be impaired thus resulting in a disturbance in blood coagulation. Various methods are available for determining prothrombin time; in our hands, the Link-Shapiro[43,44,45] modification of the Quick method has proved most satisfactory, With this procedure the normal prothrombin time is 14 to 16 seconds for the undiluted plasma and 36 to 45 seconds for diluted (1:8) plasma.*

The response of the prothrombin time to the parenteral administration of synthetic vitamin K often offers aid in differentiating between hepatogenous and extrahepatic obstructive jaundice.[46] In the early stages of the latter, there is no impairment on the part of the liver cell to produce prothrombin. The increased prothrombin time is due to the impaired absorption of vitamin K resulting from a total absence or diminution of bile salts entering the duodenum. Thus, the parenteral administration of vitamin K leads to the restoration of normal prothrombin values in over 85 per cent of such cases. In patients where the jaundice is due to damage of the parenchymal liver cells, this restoration does not occur following the injection of vitamin K.

Hippuric Acid Synthesis: Hippuric acid synthesis in man occurs in the liver by the union of the benzoate radical with glycine. When 6

* These values vary with the type of thromboplastin used. In this institution, thromboplastin manufactured by the Maltine Company, New York, is employed.

gm. of sodium benzoate is given orally, the normal liver can produce 2.5 to 3 gm. of hippuric acid within 4 hours.[47] The excretion of less than 2.5 gm. of hippuric acid in the urine in 4 hours signifies liver damage providing, of course, that renal function is not impaired.

The test may be made more sensitive by giving the sodium benzoate intravenously.[48] In this instance 1.77 gm. of sodium benzoate in 20 ml. sterile water is slowly injected intravenously. One hour later, the patient voids and the hippuric acid concentration in the urine is determined. The normal range is 0.7 to 0.95 gm. of hippuric acid.

Hippuric acid synthesis by the liver is reduced in acute and chronic parenchymal disease and in metastatic carcinoma of the liver. It is normal in obstructive jaundice provided no secondary biliary cirrhosis has resulted from longstanding obstruction.

Serum Alkaline Phosphatase: Alkaline phosphatase is an enzyme concerned with several metabolic processes. Whether the liver produces alkaline phosphatase has not been definitely established; however, this enzyme is present in the bile. In cases of obstructive jaundice (intrahepatic and extrahepatic), the serum alkaline phosphatase is elevated. Normal values for adults by the Bodansky[49,50] method range from 4 to 6 units per 100 ml. of serum. An alkaline-phosphatase above 13 units in an adult with jaundice is indicative of an obstructive lesion. It must be remembered that many other clinical entities may cause an elevation of the serum alkaline phosphatase, e.g., hyperparathyroidism, Paget's disease of bone, metastatic carcinoma to bone, etc. When properly evaluated, however, the serum alkaline phosphatase is a definite aid in the differential diagnosis of jaundice.

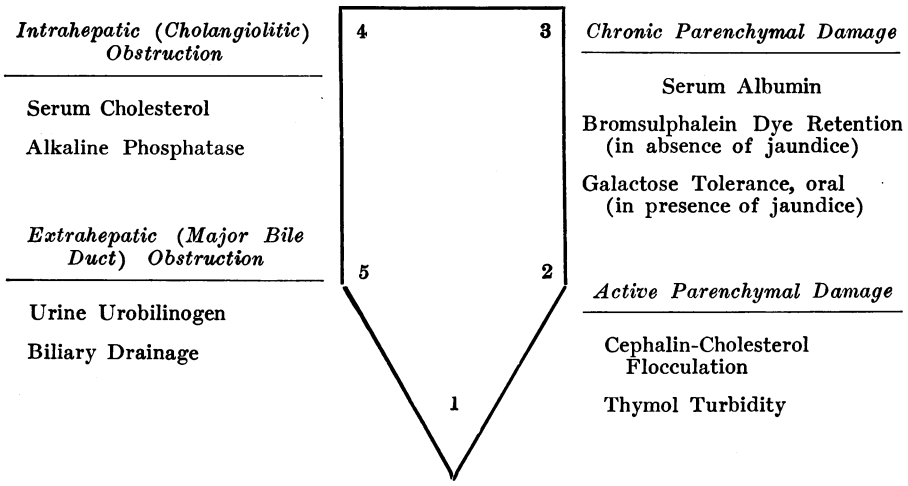
Discussion

The clinician is frequently overwhelmed by the large number of tests available for the estimation of liver function. A perusal of Table I exemplifies this state of affairs. For this reason, there is presented in Figure 1 a simple schema (modified after Greene[51]) coördinating the more important tests for estimating the functional capacity of the liver and for differentiating the various types of jaundice.

In this figure, liver and/or billiary tract disease is analyzed according to the presence or absence of five factors, namely, jaundice, active (acute) parenchymal damage, chronic parenchymal damage, intrahepatic obstruction and extrahepatic obstruction. The present authors

FIGURE 1.

SCHEMA FOR COORDINATION OF LIVER FUNCTION TESTS
(MODIFIED AFTER GREENE)



Types of Liver Disease	*Alteration in Pattern*
Hemolytic Icterus	1
Acute Hepatitis	1, 2
Cirrhosis of Liver with Jaundice	1, 2, 3
Cirrhosis of Liver without Jaundice	2, 3
Cholangiolitic Obstruction	1, 2, 4
Common Duct Obstruction	1, 4, 5
Common Duct Obstruction with Biliary Cirrhosis	1, 2, 3, 4, 5
Carcinoma of Liver (Primary or Secondary)	Irregular

have grouped these factors around a porphyrin ring because of the five points available in this configuration (peculiarly enough, the basic structure of bilirubin is a porphyrin ring). Thus, the presence of jaundice may be revealed by an elevation of the serum bilirubin and icterus index, active parenchymal damage by positive cephalin-cholesterol flocculation and thymol turbidity tests, chronic parenchymal damage by decreased serum albumin, by bromsulphalein dye retention in the absence of jaundice and by an abnormal galactose tolerance test in the presence of jaundice, intrahepatic (cholangiolitic) obstruction by an elevated serum cholesterol and alkaline phosphatase and finally, extrahepatic (major bile duct) obstruction by the persistent absence of urobilinogen in the urine and of bile in duodenal drainage. Below the figure, various types of liver disease are tabulated and the associated alterations in the pattern are indicated. Thus, in hemolytic icterus, there is jaundice as revealed by a high serum bilirubin and icterus index but no disturbances in the other four factors. In cirrhosis of the liver without jaundice only factors 2 and 3 are abnormal, namely, evidence of active parenchymal damage (positive flocculation tests) and chronic parenchymal damage (decreased serum albumin and bromsulphalein dye retention) and so on.

This schema is by no means complete or infallible. It is intended as a framework to which may be added other liver function tests depending upon the particular desires or experiences of the clinician. It possesses, however, simplicity and not infrequently affords diagnostic aid by the utilization of relatively few laboratory procedures.

Under the heading of flocculation tests, a number of procedures have been described which attempt to assay aberrations of the serum protein fractions resulting from disease of the parenchymal cells of the liver. In Table II the results obtained in five patients chosen from a large series[52] are detailed. Thus, in Case 2, a patient with infectious hepatitis, it may be noted that each of the flocculation or precipitation tests is abnormal. Case 3 is an example of the results obtained in early obstructive jaundice; each of the flocculation tests remains normal even in the presence of marked hyperbilirubinemia. When secondary biliary cirrhosis supervenes during the course of obstructive jaundice, the flocculation procedures become abnormal as may be noted in Case 4. The findings in active portal cirrhosis are shown by the results obtained in Case 5; hepatic damage is revealed by abnormal flocculation tests

TABLE II. COMPARISON OF SEVERAL FLOCCULATION TESTS IN PATIENTS WITH HEPATIC DISEASE

Case	Diagnosis	Iclerus Index units	Cephalin-Cholesterol Flocculation 24 hour reading	Thymol Turbidity units	Thymol Flocculation	Colloidal Red Flocculation	Zinc Sulphate Precipitation 1	2	3 hours	4	5
	NORMAL RANGE	4—6	0—+	0—4	0—+	0—2	0	0	0	0	0
1	Normal	6	±	1	0	0	0	0	0	0	0
2	Infectious Hepatitis	176	++++	12	++++	5	++	+++	++++	++++	++++
3	Obstructive Jaundice Due to Carcinoma of the Head of the Pancreas	121	0	1	0	0	0	0	0	0	0
4	Congenital Atresia of the Common Bile Duct with Secondary Biliary Cirrhosis	140	++	5	+++	4	0	±	+	++	+++
5	Portal Cirrhosis	24	++	5	+	5	+++	++++	++++	++++	++++
6	Familial Hepatic Dysfunction	12	+++	2	++	3	0	+	+	++	+++

throughout. In Case 6, a slight dissociation between the various procedures is demonstrated. In this patient with familial hepatic dysfunction, the thymol turbidity test alone remains normal; it may be assumed that the serum lipoproteins were not affected by the underlying hepatic dysfunction.

It appears worthwhile to stress again here that these simple though important laboratory procedures supplement each other. When these flocculation tests are carried out simultaneously, a better panorama of the alteration in the serum protein fractions is envisaged than can possibly be obtained by the performance of any single procedure alone.

Summary

1. A review and discussion of the practical liver function tests available to-day are presented.

2. The newer flocculation tests are described and compared.

3. A simple schema is presented coördinating several liver function tests as an aid in the diagnosis of diseases of the liver and biliary tract.

REFERENCES

1. Greene, C. H. and Bruger, M. The functional study of the liver and its clinical evaluation, *New York State J. Med.*, 1943, *43*:318.
2. Thannhauser, S. J. and Andersen, E. Methodik der quantitativen Bilirubinbestimmung in menschlichen Serum, *Deutsches Arch. f. klin. Med.*, 1921, *137*:179.
3. Malloy, H. T. and Evelyn, K. A. The determination of bilirubin with a photoelectric colorimeter, *J. Biol. Chem.*, 1937, *119*:481.
4. Ducci, H. and Watson, C. J. The quantitative determination of the serum bilirubin with special reference to the prompt reacting and the chloroform soluble types, *J. Lab. & Clin. Med.*, 1945, *30*:293.
5. Gellis, S. S. and Stokes, J., Jr. The methylene blue test in infectious (epidemic) hepatitis, *J. A. M. A.*, 1945, *128*:782.
6. Stokes, G. D., Gambill, E. E. and Osterberg, A. E. The methylene blue test for bilirubinuria: clinical and spectrophotometric observations, *J. Lab. & Clin. Med.*, 1946, *31*:924.
7. Watson, J., Meads, M. and Castle, W. B. Tests for urinary bilirubin, *J. A. M. A.*, 1945, *128*:308.
8. Godfried, E. G. Clinical tests for bilirubin in urine, *Biochem. J.*, 1934, *28*: 2056.
9. Hawkinson, V., Watson, C. J. and Turner, R. H. A modification of Harrison's test for bilirubin in the urine, *J. A. M. A.*, 1945, *129*:514.
10. Watson, C. J., Schwartz, D., Sborov, V. and Bertie, E. Studies of urobilinogen; a simple method for the quantitative recording of the Ehrlich reaction as carried out with urine and feces, *Am. J. Clin. Path.*, 1944, *14*:605.
11. Rosenthal, S. M. and White, E. C. Clinical application of the bromsulphalein test for hepatic function, *J. A. M. A.*, 1925, *84*:1112.
12. O'Leary, P. A., Greene, C. H. and Rowntree, L. G. Diseases of the liver; the various types of syphilis of the liver with reference to tests for hepatic

function, *Arch. Int. Med.*, 1929, *44*:155.

13. Mateer, J. G., Baltz, J. I., Marion, D. F. and MacMillan, J. M. Liver function tests: general evaluation of liver function tests and appraisal of comparative sensitivity and reliability of newer tests with particular emphasis on cephalin-cholesterol flocculation test, intravenous hippuric acid test and improved bromsulphalein test with new normal standard, *J A. M. A.*, 1943, *121*:723.
14. Bauer, R. Weitere Untersuchungen über alimentäre Galaktosurie, *Wien. med. Wchnschr.*, 1906, *66*:2537.
15. Bensley, E. H. The galactose tolerance test as an aid to diagnosis in jaundice, *Canad. M. A. J.*, 1935, *33*:360.
16. Bassett, A. M., Althausen, T. L. and Coltrin, G. C. A new galactose test for differentiation of obstructive from parenchymatous jaundice, *Am. J. Digest. Dis.*, 1941, *8*:432.
17. Bloor, W. R. and Knudson, A. The separate determinations of cholesterol and cholesterol esters in a small amount of blood, *J. Biol. Chem.*, 1916, *27*:107.
18. Thannhauser, S. J. and Schaber, H. M. Ueber die Beziehungen des gleichgewichtes Cholesterin und Cholesterinester im Blut und Serum zur Leberfunktion, *Klin. Wchnschr.*, 1926, *5*:252.
19. Epstein, E. Z. and Greenspan, E. B. Clinical significance of the cholesterol partition of the blood plasma in hepatic and in biliary diseases, *Arch. Int. Med.*, 1936, *58*:860.
20. Hurxthal, L. M. and Hunt, H. M. Clinical relationships of blood cholesterol with summary of our present knowledge of cholesterol metabolism, *Ann. Int. Med.*, 1935, *9*:717.
21. Klein, W. Ueber die enzymatische Hydrolyse der Cholesterinester des menschlichen Serums, *Ztschr. f. physiol. Chem.*, 1938, *254*:1.
22. Greene, C. H., Hotz, R. and Leahy, E. Clinical value of determination of cholesterol esters of blood in hepatic disease, *Arch. Int. Med.*, 1940, *65*:1130.
23. Tumen, H. J. and Bockus, H. L. The clinical significance of serum proteins in hepatic diseases compared with other liver function tests, *Am. J. M. Sc.*, 1937, *193*:788.
24. Post, J. and Patek, A. J. Serum proteins in cirrhosis of the liver; relation to prognosis and formation of ascites, *Arch. Int. Med.*, 1942, *69*:67.
25. Hanger, F. M. The flocculation of cephalin-cholesterol emulsions by pathological sera, *Tr. A. Am. Physicians*, 1938, *53*:148.
26. Wade, L. J. and Richman, E. H. The cephalin-cholesterol flocculation test, *J. Lab. & Clin. Med.*, 1945, *30*:6.
27. Lippman, R. W. and Bakst, H. Clinical use of cephalin-cholesterol flocculation test, *J. Lab. & Clin. Med.*, 1942, *27*:777.
28. Guttman, S. A., Potter, H. R., Hanger, F. M., Moore, D. B., Pierson, P. S. and Moore, D. H. Significance of cephalin-cholesterol flocculation test in malarial fever, *J. Clin. Investigation*, 1945, *24*:296.
29. DeJong, H. and St. John, J. H. The cephalin-cholesterol flocculation test in catatonic—and other schizophrenics, *J. Nerv, & Ment. Dis.*, 1945, *101*:572.
30. Kabat, E. A., Hanger, F. M., Moore, D. H. and Landow, H. The relation of cephalin flocculation and colloidal gold ractions to the serum proteins, *J. Clin. Investigation*, 1943, *22*:563.
31. Moore, D. B., Pierson, P. S., Hanger, F. M. and Moore, D. H. Mechanism of the positive cephalin-cholesterol flocculation reaction in hepatitis, *J Clin. Investigation*, 1945, *24*:292.
32. Maclagan, N. F. The thymol turbidity test as an indicator of liver dysfunction, *Brit. J. Exper. Path.*, 1944, *25*:234.
33. Recant, L., Chargaff, E. and Hanger, F. M. Comparison of the cephalin flocculation with the thymol turbidity test, *Proc. Soc. Exper. Biol. & Med.*, 1945, *60*:245.
34. Watson, C. J. and Rappaport, E. M. A comparison of the results obtained with the Hanger cephalin-cholesterol flocculation test and the Maclagan thymol turbidity test in patients with liver disease, *J. Lab. & Clin. Med.*, 1945, *30*: 983.
35. Kunkel, H. G. and Hoagland, C. L. Mechanism and significance of the thy-

mol turbidity test for liver disease, *J. Clin. Investigation,* 1947, *26:*1060.

36. Linder, H. K., Bruger, M. and Greene, C. H. Comparative studies with some newer tests for hepatic dysfunction: thymol turbidity, cephalin-cholesterol flocculation and colloidal gold reaction, *New York State J. Med.,* 1948, *48:*1371.
37. Shank, R. E. and Hoagland, C. L. A modified method for the quantitative determination of the thymol turbidity reaction of the serum, *J. Biol. Chem.,* 1946, *162:*133.
38. Ley, A. B., Lewis, J. H. and Davidson, C. S. The quantitative determination of the thymol turbidity reaction of serum, *J. Lab. & Clin. Med.,* 1946, *31:* 910.
39. Kingsbury, F. B., Clark, C. P., Williams, G. and Post, A L. The rapid determination of albumin in urine, *J. Lab. & Clin. Med.,* 1926, *11:*981.
40. Neefe, J. R. Results of hepatic tests in chronic hepatitis without jaundice, *Gastroenterology,* 1946, *7:*1.
41. Ducci, H. The colloidal red test for the study of hepatic dysfunction, *J. Lab. & Clin. Med.,* 1947, *32:*1273.
42. Kunkel, H. G. Estimation of alterations of serum gamma globulin by a turbidimetric technique, *Proc. Soc. Exper. Biol. & Med.,* 1947, *66:*217.
43. Campbell, H. A., Smith, W. K., Roberts, W. L. and Link, K. P. Studies on hemorrhagic sweet clover disease; bioassay of hemorrhagic concentrates by following prothrombin level in plasma of rabbit blood, *J. Biol. Chem.,* 1941, *138:*1.
44. Shapiro, S., Sherwin, B., Redish, M. and Campbell, H. A. Prothrombin estimation; procedure and clinical interpretations, *Proc. Soc. Exper. Biol. & Med.,* 1942, *50:*85.
45. Shapiro, S. Hyperprothrombinemia, premonitory sign of thrombo-embolization (description of method), *Exper. Med. & Surg.,* 1944, *2:*103.
46. Lord, J. W., Jr. and Andrus, W. deW. Differentiation of intrahepatic and extrahepatic jaundice, *Arch. Int. Med.,* 1941, *68:*199.
47. Quick, A. J. The synthesis of hippuric acid; a new test of liver function, *Am. J. M. Sc.,* 1933, *185:*630.
48. Quick, A. J. Intravenous modification of the hippuric acid test of liver function, *Am. J. Digest. Dis.,* 1939, *6:*716.
49. Bodansky, A. Determination of serum phosphatase. Factors influencing the accuracy of the determination, *J. Biol. Chem.,* 1933, *101:*93.
50. Bodansky, A. Notes on determination of serum inorganic phosphate and serum phosphatase activity, *Am. Clin. Path.* (Tech. Supp.), 1937, *7:*51.
51. Greene, C. H. *Personal communication.*
52. Oppenheim, E., Bruger, M. and Frost, E. *Unpublished data.*